P2-152 MECHANISM UNDERLYING APOLIPOPROTEIN E (APOE) ISOFORM-DEPENDENT HDL GENERATION AND IMPAIRMENT OF APOE3-MEDIATED HDL GENERATION BY HOMOCYSTEINE

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Background: We determined the molecular mechanisms underlying apolipoprotein E (ApoE)-isoform-dependent lipid efflux from neural cells, and how homocystein involves in ApoE-mediated lipid efflux in culture. **Methods:** Recombinat ApoE3, ApoE4, and their fragments were added into the primarily cultred neurons and astrocytes and lipid efflux released from these cells were determined. Astrocyte cultres from ApoE3- and ApoE4-KI mice were also used. The CSF from patients with AD, hyperhomocysteinemia, or normal control was analysed. **Results:** The ability of ApoE3 to induce lipid efflux was 2.5-3.9-fold greater than that of ApoE4. The amino-terminal fragment of ApoE3 (22-kDa-ApoE3) induced greater lipid efflux (HDL generation) than that of 22-kDa-ApoE4. Dimerization of 22-kDa- or intact-ApoE3 by disulfide binding induces a greater lipid efflux than that by monomeric ApoE3s or ApoE4s that have no cysteine and therefore remain as monomers. Addition of segments of the carboxyl-terminal domain to 22-kDa-ApoE3 additively induced lipid efflux in a length-dependent manner; in contrast, this effect did not occur with ApoE4, owing to the interaction between the amino- and carboxyl-terminal domains in ApoE4. Preincubation of ApoE3 with homocysteine inhibits its dimerization, and subsequently decreases its ability to induce HDL generation. The ratio of dimeric ApoE3 to monomeric ApoE3 in CSF from patients with hyperhomocysteinemia, which is another risk factor for Alzheimer's disease, is lower than that in CSF from normal subjects. **Conclusions:** These results suggest that two different risk factors, ApoE epsilon 4 and hyperhomocysteinemia, may share a common pathway, that is, both factors are related to the reduced level of HDL generation, leading to the development of Alzheimer's disease.

P2-153 CONTROL OF GENES TRANSCRIPTION INVOLVED IN NEURONAL LIPIDS METABOLISM BY THE AMYLOID PRECURSOR PROTEIN APP

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Background: The catabolic pathways of APP producing Aβ peptides was intensively studied in different cell types expressing human APP. However, the primary function of APP remains still unknown. A potential link between lipids and Alzheimer disease (AD) was established with ApoE, a major lipids carrier in the brain. In families with late-onset AD, the risk of AD increased from 20% to 90% with the ϵ4 allele of the ApoE gene. Therefore, cholesterol and ApoE genotype seem to interact to influence progression of AD. Many data have previously shown that APP regulates the transcription of genes. In this line of evidence, our recent results identified a potential role of APP in cholesterol homeostasis through the control of genes transcription involved in the neuronal lipids metabolism. **Methods:** Human APP (hAPP) was expressed in rat cultured of cortical neurons using recombinant adenoviruses. The amount of free and neo-synthesized cholesterol were quantified from cell lysates after ^{14}C radiolabelling. We monitored the transcription of LDL receptor and HMGCoA reductase genes by quantitative real time RT-PCR. The cleavage of SREBP, a sterol regulatory element binding protein controlling the expression of these two cholesterol target genes, was analysed by western blotting. The expression of ApoE was also analysed in these cells by immunocytochemistry and qRT-PCR. **Results:** Preliminary experiments clearly showed that the cellular cholesterol synthesis decreased in neurons upon the expression of hAPP compared with non- or mock-infected controls. This was accompanied by decreased cleavage of SREBP into its transcriptionally active form and then associated with the down-regulation of the transcription of LDL receptor and HMGCoA reductase genes. Moreover, primary cultures of rodent neurons produced endogenous ApoE. However, its expression was decreased in the neighbouring neurons which did not express hAPP and in which the metabolism of cholesterol was probably down-regulated. **Conclusions:** The present findings indicate that APP affects neuronal cholesterol homeostasis, by controlling the transcription of target genes involved in the homeostasis of lipids.

P2-154 IMPACT OF APOE GENOTYPE ON CIRCULATING IL-18 AND AMYLOID BETA LEVELS IN ALZHEIMER'S DISEASE

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Background: Alzheimer's disease (AD) is characterized by accumulation of neurofibrillary tangles, amyloid deposition, consistent deficit in cholinergic neurotransmission and dysregulation of the immune response. IL-18 has a potent immunoregulatory activity on both innate and adaptive responses. Its involvement in ageing and in neurodegenerative diseases has also been reported. The purpose of this study was to determine IL-18 and Aβ levels in patients with AD compared with healthy subjects (HC) and what effect AChEI treatment has on these levels. The association between these molecules and ApoE4 allele were also evaluated. **Methods:** 20 AD patients with clinical diagnosis according to NINCDS-ADRDA (MMSE score $\leq$18) and 10 not demented HC subjects, frequency matched for sex and age were enrolled. Plasma was collected on day of enrolment for both group and 30 days after AchEI therapy for AD patients. Levels of IL-18 and Aβ were measured with commercially available ELISA kits. INNO-LiPA ApoE is used for the identification of apolipoprotein E genotypes ϵ2, ϵ3, and ϵ4. **Results:** Plasma concentrations of IL-18 in AD patients were higher, whereas levels of Aβ were lower when compared with HC subjects. In the AD group, both ApoE4 non-carriers and ApoE4 carriers exhibited higher IL-18 levels than HC group. When comparing ApoE4 carriers to non-carriers, in the AD group we found that ApoE4 carriers had higher plasma IL-18 and Aβ levels than non-carriers. Treatment with AchEI had no significant effect on mean plasma IL-18 and Aβ concentration at 30 days of administration. **Conclusions:** Our results showed an increased production of the proinflammatory cytokines IL-18 in AD patients indicating an alteration in the cytokine profile in AD implying that inflammation plays a role in the pathophysiology of AD. One interpretation of the lower mean plasma Aβ observed in AD group is that amyloid is sequestered in senile plaques, as has been suggested for the lower levels of Aβ in the CSF of AD patients. The presence of intra- and inter-person variability warrant further investigation.

P2-155 APOE4 MAY CONTRIBUTE TO CHOLESTEROL RETENTION IN ALZHEIMER BRAIN

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Background: Alzheimer's disease (AD) is characterized by overproduction and deposition of Aβ peptides in the brain. However, the mechanism of Aβ overproduction in late onset of AD is largely uncharacterized. It is known that apoE4 allele is associated with a high risk of AD and that apolipoprotein E is involved in the metabolism and redistribution of lipids and cholesterol in the brain. Recent studies have suggested a role of altered cholesterol metabolism in Alzheimer's pathogenesis. Aβ is a product derived from sequential cleavage of APP by β-and γ-secretases. Since β-and γ-secretases are located